AD_____

Award Number: W81XWH-06-1-0694

TITLE: The SDF1-CXCR4 Axis Functions through p38-MAPK Signaling to Drive Breast Cancer Progression and Metastasis

PRINCIPAL INVESTIGATOR: Lyndsay Vanhoy

CONTRACTING ORGANIZATION: Tulane University
New Orleans, LA 70112

REPORT DATE: September 2007

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-Sep-2007	Annual Summary	30 Aug 2006 – 29 Aug 2007

4. TITLE AND SUBTITLE
The SDF-1 – CXCR4 axis functions through p38-MAPK signaling to

Drive breast cancer progression and metastasis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0694

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Lyndsay Vanhoy

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Tulane University

New Orleans, LA 70112

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
& Material Command
Fort Detrick Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The primary long-term objective of this research is to understand how chemokine signaling through MAPK influences progression of breast carcinoma cells to a hormone-independent, endocrine therapy resistant and metastatic phenotype. Our preliminary evidence demonstrates that overexpression of CXCR4 in breast carcinoma cells leads to a hormone independent phenotype in vivo. The ability of CXCR4 expression to lead to estrogen independent breast tumor growth in immunocompromised mice is correlated with CXCR4 mediated activation of downstream signaling events and enhancement of estrogen receptor mediated gene expression. We hypothesize that altered expression of CXCR4 and activation by its ligand SDF1 directly controls progression to hormone independence in breast carcinoma cells. We specifically hypothesize that the SDF1-CXCR4 axis functions through G-protein coupled signaling through the downstream MAPK pathway to regulate gene expression. Our research will provide strong evidence that combining SDF1/CXCR4 blockade with current endocrine therapy strategies will be synergistically effective in treatment for endocrine resistant and metastatic breast cancer. As a novel mechanism of hormone independence this could prove to be a unique approach to beast cancer treatment. New treatment options for patients are necessary for improved patient care.

15. SUBJECT TERMS
Breast cancer, chemokine, SDF-1

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	18	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

INTRODUCTION

The development of resistance to anti-estrogens and the progression to hormone-independence are still poorly understood problems in the treatment of ER-positive breast cancers. The function of the ER-coregulated proteins and crosstalk with ER signal pathway, growth factor mediated signaling, and other kinase networks could be responsible for this resistance. It has been shown that some chemokines lead to the initiation of migration. One such chemokine, stromal-derived growth factor 1(SDF1/CXCL12) along with its receptor chemokine X receptor 4 (CXCR4) expression is a critical component in the ability of cancer cells to invade and metastasize. It has been demonstrated that estrogen-estrogen receptor(ER) is able to mediate the upregulation of the SDF-1 thereby establishing a link between hormone and chemokine signaling in proliferation of breast carcinoma cells. This SDF1-CXCR4 axis functions to stimulate proliferation, promote cell motility/invasion, and suppress apoptosis through activation of specific downstream signaling pathways. One such pathway includes phosphatidylinositol 3-kinase (PI3K)/AKT and members of mitogen-activated protein kinase (MAPK) family such as Erk1, JNK and p38. Interestingly, both the MAPKs and PI3K-AKT have been implicated in development of resistance to endocrine therapy in breast carcinoma. It has been reported that unlike other transmembrane G-protein coupled receptors, SDF1 has the same affinity for CXCR4 in both the coupled and uncoupled state with G-protein. So the possibility exists that the interaction of SDF1 with CXCR4 can induce two distinct signal cascades, one requiring G-protein mediated signaling (our focus) and another where G-protein independent signal pathway activated through other membrane receptors. While the ability of CXCR4 or other chemokine receptors to regulate the ER has not been examined, the ability of GPCRs such as the chemokine receptor CXCR4 to regulate estrogen receptor signaling and progression of cancer cells to hormone- independence represents an important area of research. We believe our research will provide very strong evidence to resolve endocrine therapy resistance in breast cancer.

BODY

We have broken the work performed during year one of this proposal into tasks:

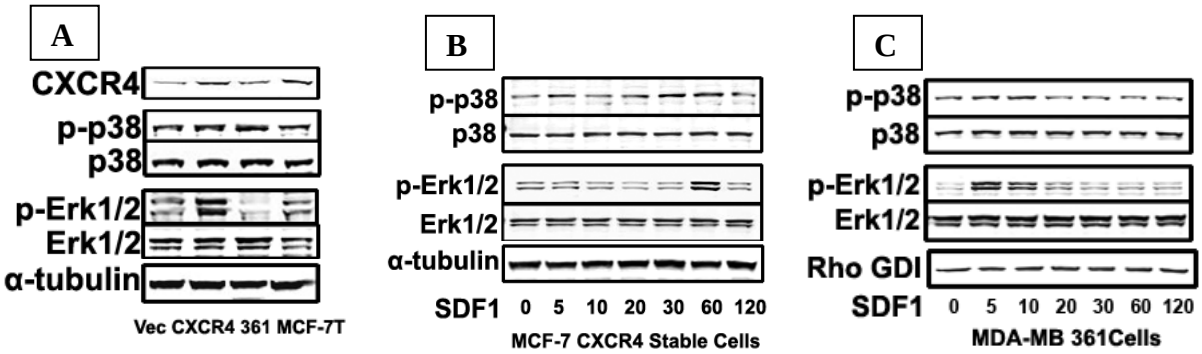
Task 1. Establish crosstalk between CXCR4/G-protein signaling and estrogen receptor (ER).

1.A Determine CXCR4 activation leads to increased ER-mediated gene expression.

Experiments to test CXCR4/SDF-1 ability to regulate ERE-luciferase acitivity in MCF-7-VEC, MCF-7-CXCR4, and MDA-MB-231 when stimulated with estrogen, Tamoxifen, or ICI have been initiated but have not produced results at this time.

A. MCF-7-vector or MCF-7-CXCR4 overexpressing stable cell lines were harvested for RNA isolation and RT-PCT to test for SDF-1 gene expression. SDF-1 is an ER regulated gene. As the figure above demonstrates, overexpression of CXCR4 induces SDF-1 expression. B. The same cell lines were treated with estrogen and harvested for RNA isolation and RT-PCR to test for ER responsive genes. Cells stimulated with estrogen exhibit an increase in SDF-1 mRNA illustrating a role for CXCR4/ER crosstalk in regulation of ER gene expression.

1.B Determine if CXCR4 activates p38.



A. Western blots of MCF-7-VEC, MCF-7-CXCR4, MDA-MB-361, MDA-MB-231for basal levels of p38 and ERK signaling. **B**. MCF-7-CXCR4 cells or **C**. MDA-MB-361 cells were treated with SDF-1 in a timecourse manner and a Western blot was performed in order to determine the phosphorylation of p38 and ERK1/2. We have shown that SDF-1 increases phosph-p38 levels in these cell lines as well as phosphor-ERK.

1.B.2 Determine the role of CXCR4 regulation of ER by p38 signaling using p38 luciferase.

This task has not yet been started. We plan to use phospho-specific ER ELISA kits for confirmation of transcription factor activation by CXCR4.

1.C Determine if p38-MAPK signaling is required for CXCR4 mediated breast cancer progression.

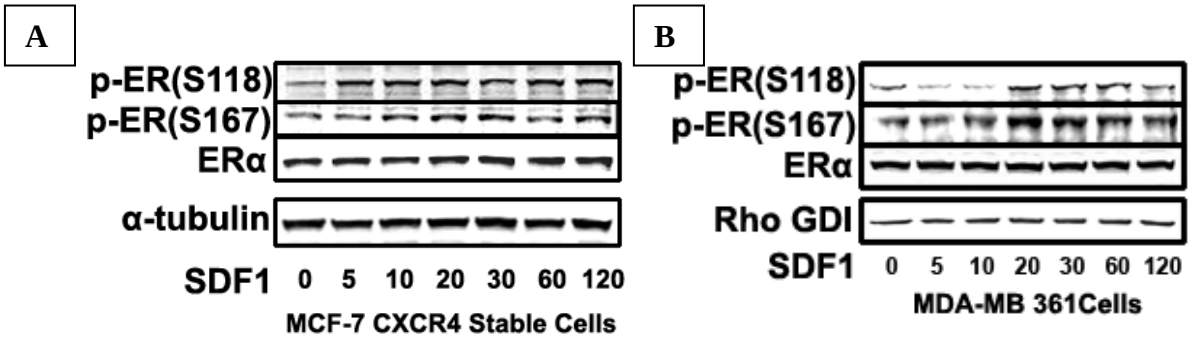
We plan to use cells treated with either shRNA to p38, chemical inhibitors of p38, or DN-p38 with ERE-luciferase to determine CXCR4 mediated gene expression. This will determine if p38-MAPK signaling is required for CXCR4 mediated cancer progression. These experiments are in progress but have not produced reportable data at this time.

Task 2. CXCR4/SDF-1 axis regulates ERE-mediated gene expression through phosphorylation of the ER.

2.A Determine which transactivation domain (AF1 or AF2) CXCR4 signaling targets on ER.

Western blots were performed to determine the total phosphorylation of ERα AF1 and AF2 sites after stimulation with SDF-1. Our first blot showed that the AF1 domain was the site of phosphorylation following treatment with SDF-1. However, subsequent blots have not been able to reproduce these results. We will not draw any conclusions as the transaction domain involved in CXCR4 signaling until we work out our antibody issues.

2.B Determine if CXCR4 phosphorylates the ER at specific sites.



Western blots were carried out on cells after treatment with and without SDF-1 using phosphor-specific antibodies to the ER. We determined that SDF-1 treated cells induce phosphorylation at both serine 118 and serine 167 on the ER in both **A.** MCF-7-CXCR4 and **B.** MDA-MB-361 cell lines.

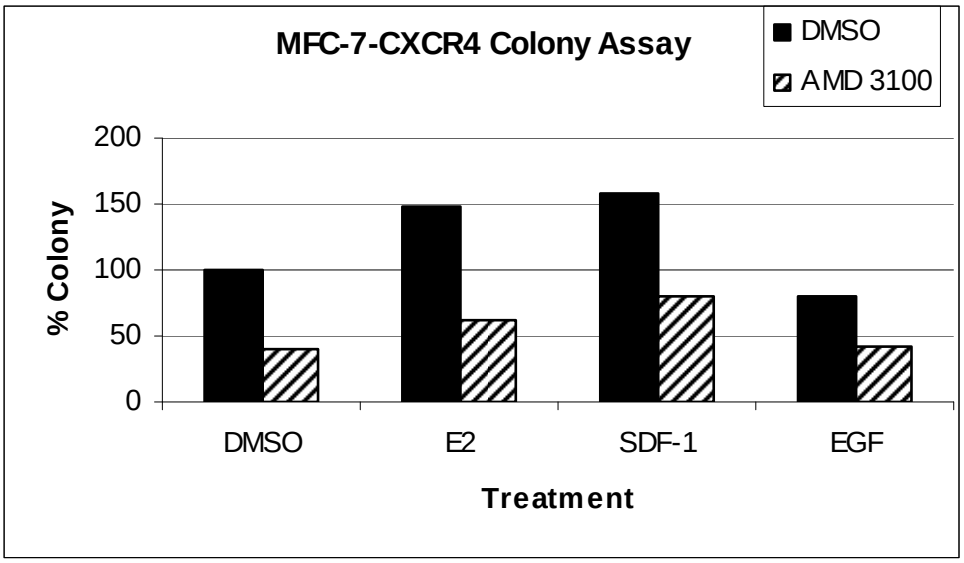
2.C Determine the requirement of ER phosphorylation by CXCR4 for mediation of gene expression.

These experiments have not yet been conducted. We plan to use phospho-mutants of the sites specified by the experiments above in conjunction with luciferase reporter assays to conclude if these sites are required and/or sufficient for ER activation. Mutants will be used alone and in combination.

Task 3. Demonstrate CXCR4/SDF-1 axis mediated gene expression is necessary for hormone independence in breast cancer.

3.A Demonstrate that hormone independence can be disrupted by blocking the CXCR4/SDF-1 axis *in vitro*.

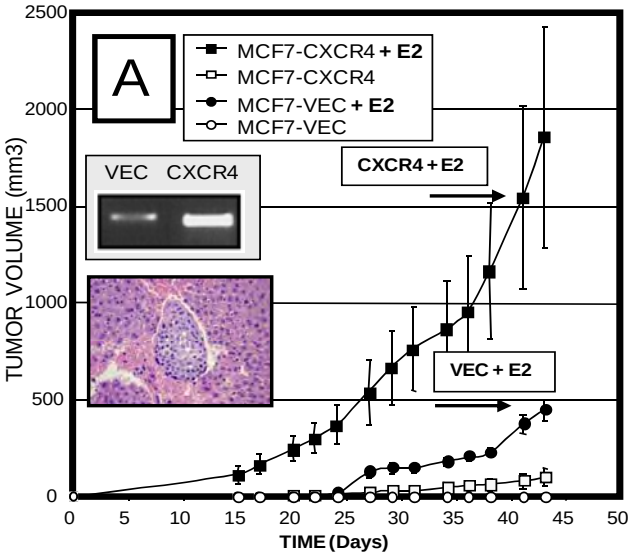
These experiments are currently in progress. Several assays have been performed using MCF-7-VEC, MCF-7-CXCR4, and MDA-MB-231 cells. We show a stimulation of colony formation with the addition of SDF-1 even in the absence of estrogen which can be knocked down with the addition of the CXCR4 specific inhibitor, AMD3100. AMD3100 also appears to inhibit colony formation in the presence of estrogen indicating a link between ER and CXCR4.



3.B Determine the role of CXCR4 expression on progression of breast cancer to invasive and metastatic phenotype dependent on ER phosphorylation *in vitro*.

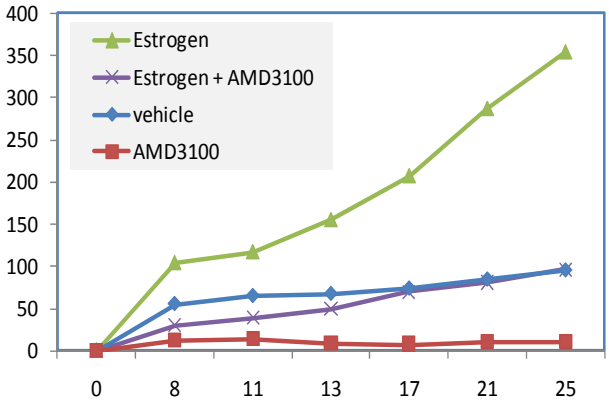
These experiments have not yet been initiated. Transwell experiments will be utilized using MCF-7-VEC, MCF-7-CXCR4, MDA-MB-361 and MDA-MB-231 cell lines treated with and without SDF-1 and/or estrogen.

3.C Test CXCR4/SDF-1 affect on tumor formation, estrogen-independence and metastasis *in vivo*.



CXCR4 expressing breast carcinoma cells exhibit hormone independent tumor formation and increased estrogen sensitivity. Female, 4-6 week old, ovariectomized SCID/beige mice (n=5/group) were injected in the MFP with 5x106 MCF7-vector or MCF7-CXCR4 cells suspended in 50ul of sterile PBS. Tumors were measured every 3 days using digital calipers.

Immunocompromised mouse models were utilized to evaluate tumor growth after injection of MCF-7-CXCR4 cells into the mammary fat pad of ovariectomized female mice with or without estrogen pellets. MCF-7-CXCR4 cells grew faster and larger than control tumors in the presence Metastasis was seen in a few of the CXCR4 mice but results were not conclusive. Livers and lungs were harvested from all mice and will be sent for sectioning to explore for metastasis.



Pharmacologic inhibition of CXCR4 suppresses estrogen stimulated breast cancer tumor growth in vivo. 5x106 MCF7-CXCR4 cells were suspended in 50ul of sterile Matrigel (reduced factor) and injected into the MFP of female ovariectomized Nude mice. A pellet of 0.72 mg, 60 day release 17-beta estradiol was implanted subcutaneously. Tumor size was monitored using digital caliper. Animals received twice daily i.p. injections of vehicle or AMD3100 (0.1mg) for 14 days.

Mice were treated with AMD3100 twice daily for 14 days. We were able to decrease the increased growth caused by overexpression of CXCR4 with the use of AMD3100 in MCF7-CXCR4 cells. Tumors were fixed in 10% formalin and have been sent off for sectioning and staining.

Similar results were seen with the use of a CXCR4 specific antibody. Anti-CXCR4 was able to decrease tumor volume in MCF7-CXCR4 cells (+/- estrogen) and MDA-MB-361 cells (+estrogen). The above data indicate that inhibition of CXCR4 is able to decrease tumor volume in vivo.

KEY RESEARCH ACCOMPLISHMENTS

- Determined CXCR4/SDF-1 increase of ER responsive genes.
- Determined CXCR4 relationship with phosphorylated p38.
- Determined SDF-1 phosphorylation of AF1 on ER.
- Determined SDF-1 specific phosphorylation at s118 on ER.
- Demonstrated SDF-1 ability to initiate increased colony formation in a hormone independent manor.
- Demonstrated CXCR4/SDF-1 ability to enhance tumorigenesis and metastasis *in vivo*.

BREAST CANCER TRAINING-RELATED OUTCOMES

- Successfully completed preliminary exam for the Biomedical Science Graduate Program.
- Lecturer, Endocrine Pharmacology class, Department of Pharmacology, Tulane University, New Orleans, LA
- Teaching assistant, Cellular and Molecular Biology Program, Tulane University, New Orleans, LA
- Mentorship, Shannon E. Muir, Pharmacology Masters student, Department of Pharmacology, Tulane University, New Orleans, LA

REPORTABLE OUTCOMES

Presentations:

"CXCR4 Drives Breast Carcinoma to Hormone-Independent and Metastatic Phenotype". Molecular and Cellular Biology Program Annual Retreat, Primate Center, Tulane University, Covington, LA. October 2006. **Lyndsay Vanhoy Rhodes**

"Chemokines in Breast Cancer". George A. Pfeiffer Science Symposium, Pfeiffer University, Misenheimer, NC. October 2006. Alumni speaker. **Lyndsay Vanhoy Rhodes**

"CXCR4 Drives Breast Carcinoma to Hormone-Independent and Metastatic Phenotype". Molecular and Cellular Biology Program Annual Research Days, Tulane University School of Medicine, New Orleans, LA. March 2007. **Lyndsay Vanhoy Rhodes**

Abstracts:

Lyndsay Vanhoy Rhodes, Alberto Salvo, Pablo Fonseca, Syreeta Tilghman, Steven Elliott, and Matthew E. Burow. "CXCR4 Drives Breast Carcinoma to Hormone-Independent and Metastatic Phenotype", Molecular and Cellular Biology Annual Research Days, Tulane University, Feb. 28 – Mar. 1, 2007, New Orleans, LA.

CONCLUSION

The hypothesis that overexpressing CXCR4 cancer cells crosstalk with ER to upregulate their own ligand (SDF1) and form a feed-forward autocrine loop which activates CXCR4 signaling constitutively provides a novel mechanism for tumor progression and metastasis. Our research will provide strong evidence that combining SDF1/CXCR4 blockade with current endocrine therapy strategies will be synergistically effective in treatment for endocrine resistant and metastatic breast cancer. As a novel mechanism of hormone independence this could prove to be a unique approach to beast cancer treatment. New treatment options for patients are necessary for improved patient care.

References:

1. Schiff R, Massarweh S, Shou J, Osborne CK. Breast cancer endocrine resistance: how growth factor signaling and estrogen receptor coregulators modulate response. Clin Cancer Res. 2003 Jan;9(1 Pt 2):447S-54S
2. Clarke R, Liu MC, Bouker KB, Gu Z, Lee RY, Zhu Y, Skaar TC, Gomez B, O'Brien K, Wang Y, Hilakivi-Clarke LA..Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling. Oncogene. 2003 Oct 20;22(47):7316-39
3. Johnston SR, Head J, Pancholi S, Detre S, Martin LA, Smith IE, Dowsett M.Integration of signal transduction inhibitors with endocrine therapy: an approach to overcoming hormone resistance in breast cancer. Clin Cancer Res. 2003 Jan;9(1 Pt 2):524S-32S.
4. Ali, S., Coombes R.C., Endocrine-Responsive Breast Cancer and Strategies for Combating Resistance. *Nature Reviews Cancer* 2, 101 -112 (2002).
5. Kiefer, T.L., Lai, L., **Burow, M.E.,** Hill, S.M. Differential regulation of estrogen receptor alpha, glucocortocoid receptor and Retinoic acid receptor transcriptional activity by melatonin is mediated via the G_{i2} protein. Submitted to *Molecular Endocrinology*.(2004)
6. Hall JM, Korach KS. Stromal cell-derived factor 1, a novel target of estrogen receptor action, mediates the mitogenic effects of estradiol in ovarian and breast cancer cells. Mol Endocrinol. 2003 May;17(5):792-803.
7. Coser KR, Chesnes J, Hur J, Ray S, Isselbacher KJ, Shioda T. Global analysis of ligand sensitivity of estrogen inducible and suppressible genes in MCF7/BUS breast cancer cells by DNA microarray. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):13994-9.
8. Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. J Biol Chem 276:9817-24, 2001.
9. Lee H, Jiang F, Wang Q, et al. 2000 MEKK1 activation of human estrogen receptor alpha and stimulation of the agonistic activity of 4-hydroxytamoxifen in endometrial and ovarian cancer cells. Mol Endocrinol 14:1882-96.
10. Driggers PH, Segars JH, Rubino DM 2001 The proto-oncoprotein Brx activates estrogen receptor beta by a P38 mitogen-activated protein kinase pathway. J Biol Chem 276:46792-7.
11. Lee H, Bai W 2002 Regulation of estrogen receptor nuclear export by ligand-induced and P38-mediated receptor phosphorylation. Mol Cell Biol 22:5835-45
12. Frigo, D.E., Simpson E.N., Weldon, C.B., **Elliott, S**., Melnik, L.I., Dugan, C.B., Collins-Burow, B.M., Zhu, Y., **Salvo, V.A.,** Lopez, G.N., Kushner, P.J., Curiel, T.J., McLachlan, J.A., **Burow, M.E.** The P38 MAPK Stimulates Estrogen-Mediated Transcription and Proliferation Through the Phosphorylation and Potentiation of the p160 Coactivator GRIP1. *Submitted* (2004)
13. Katzenellenbogen BS Estrogen receptors: bioactivities and interactions with cell signaling pathways. *Biol Reprod* 54:287-93, 1996.
14. Kato S.Estrogen receptor-mediated crosstalk with growth factor signaling pathways. *Breast Cancer* 8(1): 3-9, 2001.

15. Smith CL Crosstalk between peptide growth factor and estrogen receptor signaling pathways. *Biol Reprod* 58:627-32, 1998.
16. Aronica SM, Katzenellenbogen BS Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate and insulin-like growth factor-I. *Mol Endocrinol* 7:743-752, 1993.
17. Ignar-Trowbridge DM, Nelson KG, Bidwell MC, Curtis SW, Washburn TF, McLachlan JA, Korach KS. Coupling of dual signaling pathways: epidermal growth factor action involves the estrogen receptor. *Proc Natl Acad Sci U S A* 89:4658-4662, 1992
18. Ignar-Trowbridge DM, Pimentel M, Parker MG, McLachlan JA, Korach KS Peptide growth factor crosstalk with the estrogen receptor requires the A/B domain and occurs independently of protein kinase C or estradiol. *Endocrinology* 137:1735-1744, 1996.
19. Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, and Chambon P. Activation of the Estrogen Receptor Through Phosphorylation by Mitogen-Activated Protein Kinase. *Science* 270:1491-1494, 1995.
20. Bunone G, Briand PA, Miksicek RJ, Picard D Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO J* 15:2174-83, 1996
21. Nanki, T. & Lipsky, P.E. Cutting edge: stromal cell-derived factor-1 is a costimulator for CD4+ T cell activation. *J Immunol* 164, 5010-4 (2000).
22. Zhou Y, Larsen PH, Hao C, Yong VW. CXCR4 is a major chemokine receptor on glioma cells and mediates their survival. J Biol Chem. 2002 Dec 20;277(51):49481-7. Epub 2002 Oct 17.
23. Juarez J, Bendall L.SDF-1 and CXCR4 in normal and malignant hematopoiesis. Histol Histopathol. 2004 Jan;19(1):299-309.
24. Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, Bernasconi S, Saccani S, Nebuloni M, Vago L, Mantovani A, Melillo G, Sica A. Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med. 2003 Nov 3;198(9):1391-402.
25. Salcedo R, Oppenheim JJ.Role of chemokines in angiogenesis: CXCL12/SDF-1 and CXCR4 interaction, a key regulator of endothelial cell responses. Microcirculation. 2003 Jun;10(3-4):359-70.
26. **Zou W**, Machelon V, Coulomb-L'Hermin A, Borvak J, Nome F, Isaeva T, Wei S, Krzysiek R, Durand-Gasselin I, Gordon A, Pustilnik T, Curiel DT, Galanaud P, Capron F, Emilie D, **Curiel TJ**. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med.* 7:1339-46, 2001.
27. Mohle, R. et al. The chemokine receptor CXCR-4 is expressed on CD34+ hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. *Blood* 91, 4523-30. (1998).
28. Geminder, H. et al. A possible role for CXCR4 and its ligand, the CXC chemokine stromal cell-derived factor-1, in the development of bone marrow metastases in neuroblastoma. *J Immunol* **167**, 4747-57 (2001).

29. Scotton CJ, Wilson JL, Scott K, Stamp G, Wilbanks GD, Fricker S, Bridger G, Balkwill FR.Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. Cancer Res. 2002 Oct 15;62(20):5930-8.
30. Taichman, R.S. et al. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res* 62, 1832-7 (2002).
31. Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM.The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. Am J Respir Crit Care Med. 2003 Jun 15;167(12):1676-86.
32. Muller, A. et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410, 50-6. (2001).
33. Zeelenberg, I.S., Ruuls-Van Stalle, L. & Roos, E. Retention of CXCR4 in the endoplasmic reticulum blocks dissemination of a T cell hybridoma. *J Clin Invest* **108**, 269-77 (2001).
34. Sehgal, A., Keener, C., Boynton, A.L., Warrick, J. & Murphy, G.P. CXCR-4, a chemokine receptor, is overexpressed in and required for proliferation of glioblastoma tumor cells. *J Surg Oncol* **69**, 99-104. (1998).
35. Koshiba, T. et al. Expression of stromal cell-derived factor 1 and CXCR4 ligand receptor system in pancreatic cancer: a possible role for tumor progression. *Clin Cancer Res* **6**, 3530-5. (2000).
36. Rempel, S.A., Dudas, S., Ge, S. & Gutierrez, J.A. Identification and localization of the cytokine SDF1 and its receptor, CXC chemokine receptor 4, to regions of necrosis and angiogenesis in human glioblastoma. *Clin Cancer Res* **6**, 102-11. (2000).
37. Scotton, C.J. et al. Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res* **62**, 5930-8 (2002).
38. Scotton, C.J., Wilson, J.L., Milliken, D., Stamp, G. & Balkwill, F.R. Epithelial cancer cell migration: a role for chemokine receptors? *Cancer Res* **61**, 4961-5 (2001).
39. Rubin JB, Kung AL, Klein RS, Chan JA, Sun Y, Schmidt K, Kieran MW, Luster AD, Segal RA.A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. Proc Natl Acad Sci U S A. 2003 Nov 11;100(23):13513-8.
40. Ganju RK, Brubaker SA, Meyer J, Dutt P, Yang Y, Qin S, Newman W, Groopman JE.The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. J Biol Chem. 1998;273(36):23169-75.
41. Vila-Coro AJ, Rodriguez-Frade JM, Martin De Ana A, Moreno-Ortiz MC, Martinez-A C, Mellado M.The chemokine SDF-1alpha triggers CXCR4 receptor dimerization and activates the JAK/STAT pathway. FASEB J. 1999 Oct;13(13):1699-710.
42. Vlahakis SR, Villasis-Keever A, Gomez T, Vanegas M, Vlahakis N, Paya CV.G protein-coupled chemokine receptors induce both survival and apoptotic signaling pathways. J Immunol. 2002 Nov 15;169(10):5546-54.

43. Roland J, Murphy BJ, Ahr B, Robert-Hebmann V, Delauzun V, Nye KE, Devaux C, Biard-Piechaczyk M. Role of the intracellular domains of CXCR4 in SDF-1-mediated signaling. Blood. 2003 Jan 15;101(2):399-406.
44. Sun Y, Cheng Z, Ma L, Pei G.Beta-arrestin2 is critically involved in CXCR4-mediated chemotaxis, and this is mediated by its enhancement of p38 MAPK activation. J Biol Chem. 2002 Dec 20;277(51):49212-9.
45. Baldwin WS, Curtis SW, Cauthen CA, Risinger JI, Korach KS, Barrett JC.BG-1 ovarian cell line: an alternative model for examining estrogen-dependent growth in vitro. In Vitro Cell Dev Biol Anim. 1998, 34(8):649-54.
46. Levenson AS, Jordan VC.MCF-7: the first hormone-responsive breast cancer cell line. Cancer Res. 1997 Aug 1;57(15):3071-8.
47. **Burow, ME**, Weldon, CB, Tang Y, McLachlan, JA, **Beckman, BS**. Oestrogen-mediated suppression of TNF-induced apoptosis in MCF7 cells: subversion of Bcl-2 by anti-oestrogens. *J Steroid Biochem. & Mo Biol.* 78:409-418, 2001.
48. Simstein R, **Burow M.E.,** Parker A, Weldon C.B., **Beckman B.S.** Apoptosis, chemoresistance, and breast cancer: insights from the MCF7 cell model system Exp Biol Med (Maywood). 2003 Oct;228(9):995-1003
49. Frigo, D.E., Tang, Y., **Beckmans, B.S.,** Scandurro,A.B., Alam, J., **Burow, M.E.,** McLachlan, J.A. Mechanism of AP-1-mediated gene expression by select organochlorines through the P38 MAPK pathway. *Carcinogenesis.* (2004). 25(2): 249-261, (2004).
50. Alam, J., Dunne, C., Stewart, D., Touchard, C., Otterbein, S., Choi, A.M.K., **Burow, M.E.,** Tou J-S. Mechanism of Heme Oxygenase-1 Gene Activation by Cadmium in MCF7 Mammary Epithelial Cells. *J. Biol. Chem*. 275(36): 27694-27702 (2000).
51. Buteau-Lozano H, Ancelin M, Lardeux B, Milanini J, Perrot-Applanat M. Transcriptional regulation of vascular endothelial growth factor by estradiol and tamoxifen in breast cancer cells: a complex interplay between estrogen receptors alpha and beta. Cancer Res. 2002 Sep 1;62(17):4977-84.
52. Sengupta K, Banerjee S, Saxena N, Banerjee SK. Estradiol-induced vascular endothelial growth factor-A expression in breast tumor cells is biphasic and regulated by estrogen receptor-alpha dependent pathway. Int J Oncol. 2003 Mar;22(3):609-14.
53. Weldon, C.B., **Elliott, S.,** Zhu, Y., Clayton, J.L., **Curiel, T.J**., Jaffe, B.M., **Burow, M.E**. Regulation of Estrogen-Mediated Cell Survival and proliferation by p160 Coactivators. Submitted to *Surgery* (2004).
54. Struckhoff, A.P., Bittman, R., **Burow M.E.,** Clejan, S., Elliott, S., Hammond, T., Tang, Y., **Beckman, B.S**. Novel Ceramide Analogues as Potential Chemotherapeutic Agents in Breast Cancer. *J. Pharmacol. Experimental Therapeutics*. (2004). In press
55. Weldon, C.B., Parker, A.P. Patten, D., **Elliot S.,** Tang, Y., Frigo, D.E., Dugan, C.M., Coakley, E.L., Butler, N.N., Clayton, J.L., Alam, J., **Curiel, T.J., Beckman, B.S.,** Jaffe, B.M., **Burow, M.E**. Sensitization of Apoptotically-Resistant Breast Carcinoma Cells to TNF and TRAIL by Inhibition of P38 Mitogen-Activated Protein Kinase Signaling. *International Journal of Oncology* 24: 0-00 (2004). In press

56. **Curiel, T.J.,** Wei, S., Dong, H., Alvarez, X., Cheng, P., Mottram, P., Krzysiek, R., Knutson, K.L., Daniel, B., Zimmermann, M.C., David, O., **Burow, M.E.,** Gordon, A., Dhurandhar, N., Myers, L., Berggren, R., Hemminki, A., Alvarez, R.D., Emilie, D., Curiel, D.T., Chen, L., **Zou, W.** Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nature Medicine* 9(5): 562-567, (2003)
57. Ballif BA, Blenis J. Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. *Cell Growth Differ.* 12:397-408, 2001.
58. **Burow ME,** Weldon CB, Collins-Burow BM, Ramsey N, McKee A, Klippel A, McLachlan JA, Clejan S., **Beckman BS** Crosstalk between phosphatidylinositol-3 kinase and sphingomyelinase pathways as a mechanism for cell survival/death decisions. *J. Biol. Chem.* 275:9628-9635, 2000.
59. **Burow ME,** Weldon CB, Melnik L, Duong B, Collins-Burow BM, Klippel A, **Beckman BS**, McLachlan JA. Phosphatidylinositol 3-kinase/Akt mediated regulation of NF-κB signaling events as a mechanism for suppression of TNF-induced apoptosis. *Biochem. Biophys. Res. Comm*. 271:342-345, 2000.
60. Weldon, C.B., **Burow, M.E.,** Rolfe, K.W., Clayton J.L., Jaffe B.M., **Beckman, B.S.** NF-κB-mediated chemoresistance in breast cancer cells. *Surgery.* 130:143-150, 2001
61. Frigo, D.E., Duong, B.N., Melnik, L.I., Schief, L., Collins-Burow, B.M., Pace, D.K., McLachlan, J.A., **Burow, M.E.** Flavonoid Phytochemicals Regulate Activator Protein-1 Signal Transduction Pathways in Endometrial and Kidney Stable Cell Lines. Journal of Nutrition 132(7):1848-1853, (2002).
62. Frigo, D.E., **Burow, M.E**., Mitchell, K.A., Chiang, T-C., McLachlan, J.A. DDT and its metabolites alter gene expression in human uterine cell lines through ER-independent mechanisms. Environmental Health Perspectives 110(12):1239-1245, (2002).
63. Li, Y.M., Pan, Y., Wei, Y. Cheng, X., Zhou, B.P., Tan, M., Zhou, X., Xia, W., Hortobagyi, G.N., Yu, D., Hung, M.C. Upregulation of CXCR4 is essential for HER2-mediated tumor metastasis. *Cancer Cell*. 6(5). 459-469, (2004).
64. Bartolome, R.A., Galvez, B.G., Longo, N., Baleux, F., Muijen, G.N.P., Sanchez-Mateos, P., Arroyo, A.G., Teixido, J. Stromal Cell-Derived Factor 1α Promotes Melanoma Call Invasion across Basement Membranes Involving Stimulation of Membrane-Type 1 Matrix Metalloproteinase and Rho GTPase Activities. *Cancer Research*. 64. 2534-2543, (2004).
65. Ben-Baruch, A. Inflammatory cells, cytokines and chemokines in breast cancer progression: reciprocal tumor-microenvironment interactions. *Breast Cancer Research*. 5. 31-36. (2003).
66. Chen, S., Tuttle, D.L., Oshier, J.T., Knot, H.J., Streit, W.J., Goodenow, M.M., Harrison, J.K.. Transforming growth factor-β1 increases CXCR4 expression, stromal-derived factor-1α-stimulated signaling and human immunodeficiency virus-1 entry in human monocyte-derived macrophages. *Immunology*. 114. 565-574. (2005).
67. Kang, H., Mansel, R.E., Jiang, W.G. Genetic manipulation of stromal cell-derived factor-1 attests the pivotal role of the autocrine SDF-1-CXCR4 pathway in the

aggressiveness of breast cancer cells. *International Journal of Oncology*. 26(5). 1429-1434. (2005).

68. Vaday, G.G., Hua, S.B., Peehl, D.M., Pauling, M.H., Lin, Y.H., Zhu, L., Lawrence, D.M., Foda, H.D., Zucker, S. CXCR4 and CXCL12(SDF-1) in prostate cancer: inhibitory effects of human single chain Fv antibodies. *Clinical Cancer Research*. 10(16). 5630-5639. (2004).
69. Darash-Yahana, M., Pikarsky, E., Abramovitch, R., Zeira, E., Pal, B., Karplus, R., Beider, K., Avniel, S., Kasem, S., Galun, E., Peled, A. Role of high expression levels of CXCR4 in tumor growth, vascularization, and metastasis. *Federation of American Societies for Experimental Biology*. 18(11). 1240-1242. (2004).
70. Smith, M.C., Luker, K.E., Garbow, J.R., Prior, J.L., Jackson, E., Piwnica-Worms, D., Luker, G.D. CXCR4 regulates growth of both primary and metastatic breast cancer. *Cancer Research*. 64(23). 8604-8612. (2004).
71. Liang, Z., Yoon, Y., Votaw, J., Goodman, M.M., Williams, L., Shim, H. Silencing of CXCR4 blocks breast cancer metastasis. *Cancer Research*. 65(3). 967-971. (2005).
72. Lepteva, N., Yang, A.G., Sanders, D.E., Strube, R.W., Chen, S.Y. CXCR4 knockdown by small interfering RNA abrogates breast tumor growth in vivo. *Cancer Gene Therapy*. 12(1). 84-89. (2005).
73. Liang, Z., Wu, T., Lou, H., Yu, X., Taichman, R.S., Lau, S.K., Nie, S., Umbreit, J., Shim, H. Inhibition of breast cancer metastasis by selective synthetic polypeptide against CXCR4. *Cancer Research*. 64(12). 4302-4308. (2004).
74. Lechertier, T., Berard, M., Vassy, R., Herve, M.A., Crepin, M. Transendothelial migration of two metastatic breast carcinoma cell depend on the SDF-1-alpha-CXCR2 complexes. *Anticancer Research*. 24(6). 4011-4017. (2004).
75. Dowsland, M.H., Harvey, J.R., Lennard, T.W., Kirby, J.A., Ali, S. Chemokines and breast cancer: a gateway to revolutionary targeted cancer treatments? *Current Medical Chemistry*. 10(7). 579-592. (2003).

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Lyndsay Vanhoy Rhodes	**Graduate Student of Molecular and Cellular Biology**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Pfeiffer University, Misenheimer, North Carolina	B.S	2000 - 2004	Biology
Pfeiffer University, Misenheimer, North Carolina	B.A.	2000 - 2004	Psychology
Tulane University, New Orleans, Louisiana	Ph.D. (in progress)	2004 – current	Molecular and Cellular Biology

Research Experience:

2000 – 2004 Undergraduate Honors Independent Research Projects, Pfeiffer University, Misenheimer, NC
Antibiotic Susceptibility in Animal Products, Plasmid Isolation, Bioinformatics Program, Development of Plant Tissue Culture Protocol

2002 Teaching & Resident Assistant, Southern Piedmont Educational Consortium (SPEC), Pfeiffer University, Misenheimer, NC

2003 Research Intern, Plant Molecular and Cell Biology Program, University of Florida, Gainesville, FL

2004 Lab Assistant, James Robinson Lab, Tulane University Medical Center, New Orleans, LA

2004-2006 Teaching Assistant, Tulane University, New Orleans, LA

2005-present Graduate Student Research, Interdisciplinary Program in Molecular and Cellular Biology, Tulane University Medical Center, New Orleans, LA (Thesis Advisor: Matthew Burow)

Honors, Awards, and Other Professional Activities:

Scholarships:

2000 – 2004 Presidential Scholarship, Pfeiffer University

2000 – 2004 Medical Scholarship, Pfeiffer University

2000 – 2004 Athletic Scholarship, Varsity Swimming, Pfeiffer University

Honors:

2000 – 2004	Dean's List, Pfeiffer University
2000 – 2004	Honor Scholar, Pfeiffer University
2000 – 2004	Scholar Athlete, Pfeiffer University
2003 – Present	Psy Chi, Pfeiffer University Chapter
2004	Who's Who among American Universities and Colleges
2004 – Present	Order of the Sundial, Pfeiffer University
2005 – Present	Phi Delta Sigma, Pfeiffer University

Activities:

2003 – 2004	Vice President, Honors Colloquium, Pfeiffer University Steering Committee Student Representative, Molecular and Cellular Biology Program, Tulane University
2005 – 2006	Molecular and Cellular Biology Graduate Student Association President, Tulane University
2006 – 2007	Biological Sciences Steering Committee Student Representative, Tulane University
2006 – 2007	Biological Sciences Student Board of Representatives, Tulane University

Recent Publications:

Duong, B.N., Elliott, S., Frigo, D.E., Melnik, L.I., **Vanhoy, L.**, Tomchuck, S., Lebeau, H.P., David, O., Beckman, B.S., Alam, J., Bratton, M.R., McLachlan, J.A., Burow, M.E. AKT regulation of ER-beta transcriptional activity in breast cancer. Cancer Research. 66(17):8373-8381. (2006).

Recent Presentations:

"CXCR4 Drives Breast Carcinoma to Hormone-Independent and Metastatic Phenotype". Molecular and Cellular Biology Program Annual Retreat, Primate Center, Tulane University, Covington, LA. October 2006.

"Chemokines in Breast Cancer". George A. Pfeiffer Science Symposium, Pfeiffer University, Misenheimer, NC. October 2006. (invited Alumni speaker).

"CXCR4 Drives Breast Carcinoma to Hormone-Independent and Metastatic Phenotype". Molecular and Cellular Biology Program Annual Research Days, Tulane University School of Medicine, New Orleans, LA. March 2007. (Poster session and brief talk).

Research Support:

Current:

DoD Breast Cancer Predoctoral Traineeship Award BC061597 "The SDF1-CXCR4 Axis Functions Through p38-MAPk Signaling to Drive Breast Cancer Progression and Metastasis". My role: PI. 8/30/2006 – 9/29/2009.